

Cooperation and conflict in an insect society*

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Abstract

Cooperation and conflict are inevitable consequences whenever a group of individuals get together, be they groups of self-replicating molecules or groups of warring nations. This paper gives an overview of my research using the tropical primitively eusocial paper wasp *Ropalidia marginata*, aimed at understanding the factors that modulate cooperation and conflict in an insect society. Hamilton's rule provides an excellent theoretical framework not only for investigating the role of genetic relatedness in modulating the levels of cooperation and conflict, as has been done so extensively in the last 30 years, but also for focussing on other factors, when genetic relatedness may be relatively unimportant. Polyandry or multiple mating by queens of *R. marginata* and serial polygyny or the frequent change in queens, breakdown the genetic asymmetries created by haplodiploidy and make it genetically less advantageous to be a worker, than theoretically expected. Intra-colony kin recognition abilities appear not to be so well developed as to facilitate nepotism in the face of intra-colony genetic variability. Artificial colonies with highly elevated levels of genetic variability appear to be indistinguishable from natural colonies. We have therefore investigated the possibility that social behaviour in insect societies is at least in part mutualistic. The response of wasps to alien conspecifics in the context of their nests and outside, suggest that factors other than genetic relatedness such as inter-individual assessments, facilitated by impressive cognitive abilities, may play a significant role in modulating the levels of cooperation and conflict.

Keywords: Cooperation, conflict, insect society, kin recognition.

1. Introduction

My aim here is to provide a brief overview of some experimental and observational research programmes that have recently been completed in my laboratory, follow it up with a similar overview of other ongoing research programmes and attempt to connect them using the logical framework of cooperation and conflict in an insect society. In some ways my task is made difficult by the fact that I work in an area that is far removed from the rest of the speakers this morning. But in some ways my task is made easy because, as human beings, we are all familiar with cooperation and conflict. Indeed we are all regular participants in situations involving cooperation and conflict.

2. Cooperation and conflict

When a group of individuals get together, there is scope for both cooperation and conflict. In fact a mix of cooperation and conflict is almost inevitable; seldom is there pure coop-

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peration or pure conflict. All this is not true merely of human beings. Any group of interacting entities ranging from groups of self-replicating molecules to groups of warring nations show such a mix of cooperation and conflict. I believe that there are general principles that govern the nature of interactions between cooperation and conflict, which are fairly independent of the level of complexity of the interacting entities. For example, one might speculate that the reason for conflict is that the individual entities that make up the group retain their individual identities and the reason for cooperation is that the group has a new and collective identity beyond the individual identities. Thus the balance between cooperation and conflict would be expected to depend on the relative strengths of the individual and group identities and the extent of conflict may be expected to be correlated with the probability with which the individual entities can exist outside the group.

While my long-term goal is to investigate such potentially general principles, I have focussed on an insect society to begin my empirical and theoretical investigations. The insect societies are perhaps somewhere midway in complexity between groups of self-replicating molecules on the one hand and groups of warring nations on the other. Insect societies are enormously simpler than groups of nations and have obvious advantages for experimental investigations. On the other hand, insect societies are enormously sophisticated and rich compared to groups of replicating molecules. I hope to show that insect societies very nearly approach human societies in the richness of their interactions.

3. Insect societies

An insect society that is widely familiar is that of the honeybee. There are five major species of honeybees; the European *Apis mellifera* and four species of Asian honeybees, the common rock bee *Apis dorsata*, its Himalayan counterpart, *Apis laboriosa*, the domesticated bee *Apis cerana* and the dwarf honeybee *Apis florea* (Fig. 1). In all species, the bees live in large colonies of thousands of individuals but each colony always has a single queen who is typically the only reproductive individual in the colony. Barring a few drones who do not participate in colony maintenance, the rest of the thousands of bees are the workers—smaller female bees who spend their whole life working to rear the queen's brood. Except for laying a few unfertilized eggs in the unlikely event of the colony becoming permanently queen-less, the workers have no reproductive options. The workers are incapable of mating, starting new colonies or leading a solitary life. While there are subtle conflicts between the queen and the workers on who should produce male progeny and how much the male and female progeny should each be fed, relatively speaking the balance between cooperation and conflict is tilted decisively towards cooperation in the honeybee colony¹.

4. *Ropalidia marginata*

I therefore work with an insect society where both cooperation and conflict are equally evident. This is the old world, tropical polistine wasp *Ropalidia marginata* (Fig. 2). These wasps build small (colonies with over 100 individuals are quiet rare), open honeycomb-like nests made of paper that they manufacture with cellulose fibers scraped from



FIG. 1. *Apis florea*, the Asian dwarf honeybee. Being the most primitive of all honeybees and yet exhibiting levels of social organisation and dance communication very similar to other honeybee species, the virtually unstudied *Apis florea* is believed to hold vital secrets concerning the evolution of honeybees (Photo: R. Gadagkar).



FIG. 2. The old world tropical polistine paper wasp *Ropalidia marginata*. The simple open nests are inhabited by morphologically indistinguishable queens and workers. New colonies may be started by only one or by a group of individuals. This species is therefore an excellent model system for understanding the evolution of insect sociality (Photo: R. Gadagkar).

plants. These wasps are classified as primitively eusocial, meaning that the queen and worker castes are not morphologically differentiated. This allows a great deal of flexibility in the roles of the adult wasps. Indeed, the roles of queen and worker appear to be decided on the basis of aggressive interactions among groups of nearly equivalent individuals. More importantly, individual wasps are capable of initiating solitary nests and rearing brood to adulthood without the aid of other wasps. As in the case of the honeybees, males play no role in social life but female wasps have a number of options open to them; they may leave to found their own single foundress colonies, they may leave and join other newly initiated single or multiple foundress colonies where they may become workers or queens, they may stay back as nonreproductive workers, or they may work for some time and later drive away the queen and take over her role (Fig. 3). The nearly aseasonal tropical climate in which this species lives makes colony life possible

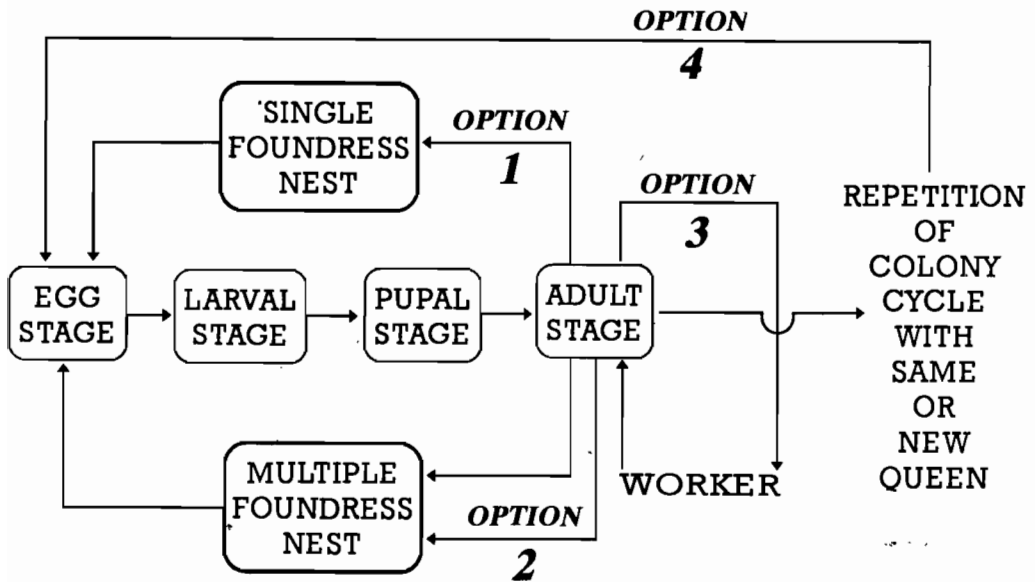


FIG. 3. The perennial indeterminate colony cycle of *Ropalidia marginata*. In the tropical aseasonal environment almost any wasp eclosing at any time of the year appears to have the option of dispersing to found solitary or multiple foundress nests, staying back and working or working for some time and then taking over as the next queen (modified from Gadagkar⁶).

throughout the year so that the various options mentioned above are available to most or all female wasps. Not surprisingly, both cooperation and conflict are conspicuously present².

Dispersing to start one's own colony is hazardous but most workers in multi-female colonies die as workers. It is therefore in the evolutionary interest of the workers to help rear the queen's brood as efficiently as possible so that they maximize their indirect or social component of inclusive fitness. But there is always a finite chance of being successful in founding or joining another colony or even of succeeding as the next queen in the present colony. Workers may also therefore be expected to be programmed by natural selection to keep their options open and not to work as hard as they might have if they had no options of their own. Given that each wasp has a certain probability of becoming a queen or a worker or of being a worker for some time and a queen for some other time, natural selection would favour wasps who maximize their life-time fitness, and that is best done by exhibiting a mixture of both cooperation and conflict.

5. The theoretical framework

The seminal papers of Hamilton^{3,4} heralded a revolution in the study of the evolution of cooperation and conflict. The key element of Hamilton's theory is that biological fitness has two components, an individual component gained by rearing offspring and an indirect

or social component gained by rearing genetic relatives. In other words, an altruistic trait will spread by natural selection if $b/c > 1/r$ where b is the benefit to the recipient, c , the cost to the actor and r , the coefficient of genetic relatedness between the actor and the recipient. Conversely, a selfish trait will spread if $b/c < 1/r$. This Hamilton's rule or inclusive fitness theory provides the framework for all modern investigations of cooperation and conflict. An inevitable consequence of using such a theoretical framework is to focus on genetic relatedness between the interacting entities as a key modulator of cooperation and conflict. Most social insects belong to the order Hymenoptera where the haplodiploid mode of sex determination creates asymmetries in genetic relatedness such that full sisters are more closely related to each other than a mother is to her offspring. This makes it possible, at least in theory, for genetic relatedness between interacting individuals to be more important than other factors in modulating levels of cooperation and conflict. All other things being equal, a wasp should be more likely to cooperate with her sister (relatedness = 0.75) than with her brother (relatedness = 0.25). All other things being equal, a wasp is better off rearing a sister than rearing her own offspring; the former is usually done by being a worker in the nest of her birth and the latter usually by leaving to found her own nest³⁻⁵.

Thus the role of genetic relatedness in moulding the evolution of cooperation and conflict has been extensively investigated for 30 years and we have now reached a stage where it is instructive to look at situations where genetic relatedness may be less important and to begin to investigate other factors which may also have a role in modulating the levels of cooperation and conflict. Notice that Hamilton's rule also provides a theoretical framework for investigating factors other than genetic relatedness; one has only to focus on the cost and benefit terms rather than merely on the relatedness term. An explicit agenda of my research has therefore been to critically examine the role of genetic asymmetries potentially created by haplodiploidy and its limitations in modulating cooperation and conflict in the primitively eusocial wasp *Ropalidia marginata*.

6. Intra-colony genetic relatedness in *R. marginata*

A factor that potentially breaks down the genetic asymmetries created by haplodiploidy is polyandry or multiple mating by the queen. If the queen mates with two or more males and simultaneously uses sperm from them then she would produce different patrilineages of daughters who would not be full sisters but would be half-sisters of each other. This can bring down the average genetic relatedness among the queen's daughters who might therefore find it more advantageous to leave and rear their own offspring rather than stay and rear half-sisters in their mother's nest. We therefore investigated mating patterns of *R. marginata* queens. Using isoenzyme polymorphism at a non-specific esterase locus, we determined the paternity of 3 to 12 daughters in four colonies. Even though we examined such a small number of daughters we found evidence of multiple mating by the queens; sperms from at least 1 to 3 males were used by the queens in producing 3 to 12 daughters⁷. The mean genetic relatedness among daughters of *R. marginata* thus turns out to be 0.53, a value not very different from a wasp's relatedness to her offspring⁸ (Table I).

Table I

Multiple mating and levels of relatedness in *Ropalidia marginata*
(modified from Gadagkar⁸)

Colony	No. of patriline	No. of daughters tested	Genetic relatedness between daughters
M11	3	10	0.48
M13	2	6	0.61
M15	3	12	0.46
M23	1	3	0.75
Weighted mean :			0.53

Nevertheless, most wasps do not leave to found their own solitary nests but rather stay on as workers. This result already points to the role of other factors, in addition to genetic relatedness, in selecting for the wasps to stay on as workers.

Another phenomenon that can potentially lower intra-colony genetic relatedness is serial polygyny or the successive presence of different queens in a colony. As mentioned above, workers can stay and work for some time and then drive away their queens and take over their roles. When this happens, workers who are daughters of one queen could be engaged in rearing brood that are the offspring of a different queen. To investigate the impact of this phenomenon on intra-colony genetic relatedness, we monitored four colonies from 37 to 86 weeks. By keeping a record of the contents of each cell and marking all wasps with unique colour codes immediately upon their eclosion, we had the entire egg, larval, pupal and adult population individually tagged for the entire duration of the study. Because there is only one queen at any given time whose identity was known at all times, we knew the genealogical relationships of all eggs, larvae, pupae and adult wasps at all times. Based on this we constructed pedigrees for the queens. These are perhaps the first-ever pedigrees for natural populations of any invertebrate (Fig. 4). The pedigrees showed that the new queens were daughters, sisters, nieces or cousins of their

Table II

Genetic relationships between successive queens and between workers and brood observed in the four colonies (modified from Gadagkar *et al.*¹⁰)

Relationship between queens and their immediate predecessors	Relationship between workers and brood
a) Daughters	1) Sisters
b) Sisters	2) Brothers
c) Nieces	3) Nieces and nephews
d) Cousins	4) Cousins
	5) Cousin's offspring
	6) Mother's cousins
	7) Mother's cousin's offspring
	8) Mother's cousin's grand offspring

Table III
Effects of serial polygyny in *R. marginata* on worker–brood genetic relatedness
 (modified from Gadagkar *et al.*¹⁰)

Colony	Number of queens	Single mating			Multiple mating (relatedness between sisters = 0.53*)	
		Relationship between successive queens	Grand mean genetic relatedness of workers to		Grand mean genetic relatedness of workers to	
			Female brood	Male brood	Female brood	Male brood
T01	4	Known	0.65	0.28	0.46	0.25
T02	5	Known	0.53	0.28	0.38	0.24
T08	10	All but one known; one unknown relationship assumed daughters	0.35	0.28	0.25	0.20
		All but one known; one unknown relationship assumed sisters	0.32	0.24	0.22	0.18
T11	2	Assumed daughters	0.63	0.29	0.45	0.26
		Assumed sisters	0.57	0.23	0.40	0.21

* Data from Muralidharan *et al.*⁷ and Gadagkar⁸.

immediate predecessor queens (Table II). However, daughters of some queens were alive even after several queen supersedures. Taking all of this into consideration, we found that the brood could be the worker's sisters and brothers, nieces and nephews, cousins, cousins' offspring, mother's cousins, mother's cousin's offspring and even mother's cousins' grand offspring (Table II). This is in stark contrast with our usual mental image of workers rearing their brothers and sisters. Based on the frequencies with which these relationships were observed and using the value of 0.53 for the average relatedness among daughters of a single mother obtained from the isoenzyme study described above, we computed mean intra-colony genetic relatedness between workers and the brood they rear. These values ranged from 0.22 to 0.46 for female brood and 0.21 to 0.26 for male brood^{9,10} (Table III). The conclusion emerged even more strongly now that if wasps generally preferred to become workers and rear female brood related by 0.22 to 0.46 and male brood related by 0.21 to 0.26 rather than initiate their own single foundress nests and rear offspring related by 0.5, then factors other than genetic relatedness must be largely responsible for tilting the cost benefit balance in favour of being a worker.

7. Can kin recognition facilitate nepotism?

Before we entirely abandon the hypothesis that asymmetries in genetic relatedness by themselves can tilt the balance in favour of the worker strategy, we must consider one

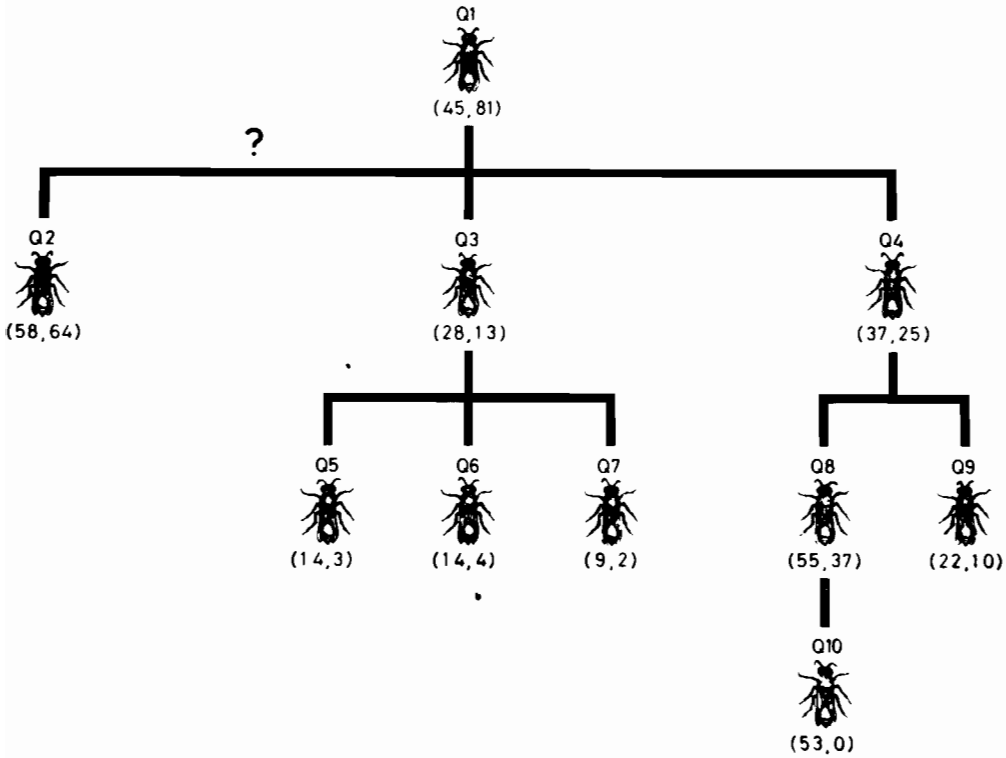


FIG. 4. A pedigree of queens in a colony of *R. marginata*. Numbers in parenthesis are tenure in days and numbers of offspring produced, in that order (modified from Gadagkar¹⁰).

additional possibility. If workers can discriminate between their full sister brood on the one hand and more distantly related brood on the other hand, and preferentially care for full sister brood, then the genetic asymmetries created by haplodiploidy and broken down by polyandry and serial polygyny, may be effectively restored. It has not been easy to test this hypothesis directly by studying feeding rates of workers towards full and half-sister larvae. We therefore began with an indirect assay. The approach we used was to study the nestmate discrimination abilities of adult wasps. When female wasps are presented with their nestmates and non-nestmates in laboratory cages outside the contexts of their nests, they show a variety of behavioural interactions towards them (Fig. 5). Using the frequencies with which different behaviours are shown and ranking the different behaviours on a tolerance scale, we constructed a tolerance index and compared tolerance shown towards nestmates and that shown towards non-nestmates. When adult wasps present on natural colonies were used, they displayed an efficient level of nestmate discrimination; nestmates were treated significantly more tolerantly than non-nestmates.

Using such an experimental assay, we showed that for efficient nestmate discrimination to occur, both the discriminating as well as the discriminated wasps should have been exposed to a fraction of their nest and a subset of their nestmates (let us call such

wasps *Exposed*). Discrimination does not occur even if one of a pair of wasps has never smelt its nest or nestmates (let us call such wasps *Isolated*) (Table IV). It is reasonable to think that wasps make discrimination by smelling something on the bodies of the encountered individuals (let us call that *Label*) and comparing it with some standard smell stored in their brains (let us call that *Template*). If an isolated wasp cannot discriminate between exposed nestmates and exposed non-nestmates then the isolated wasp must be lacking the appropriate template in its brain (because the exposed wasp is expected to have normal label and template). Similarly, if an exposed wasp cannot discriminate between the isolated nestmates and the isolated non-nestmates then the isolated wasps must be lacking the appropriate labels on their bodies. If, as we found, both the discriminating as well as the

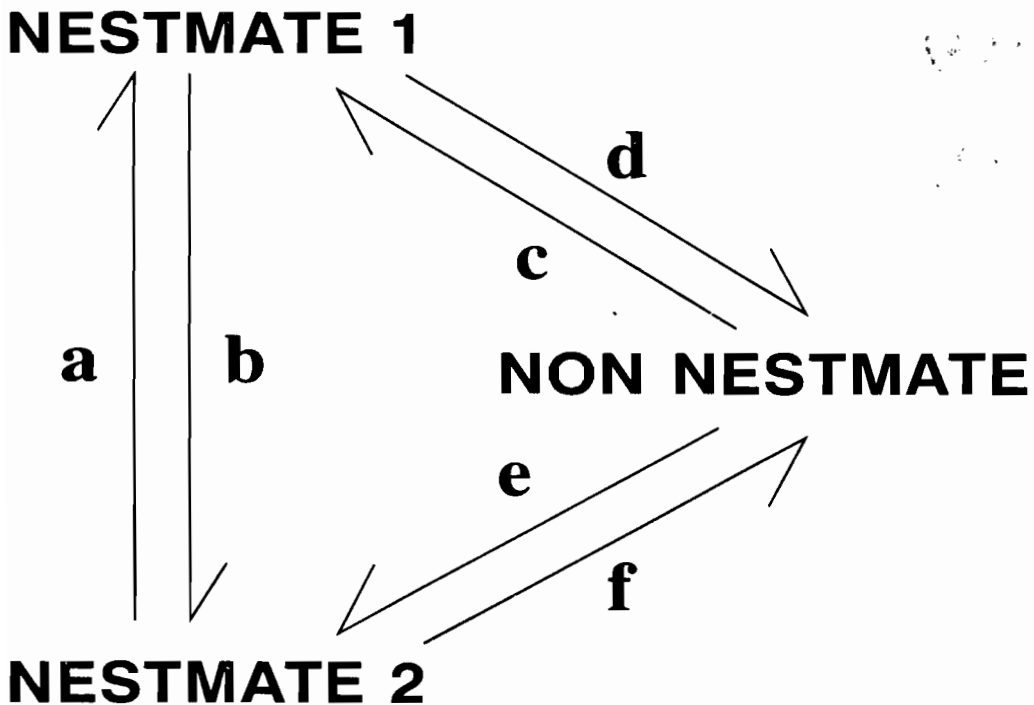
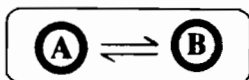


FIG. 5. The triplet assay. The behavioural interactions seen in all experiments are classified into six categories, designated *a-f*, such that all interactions initiated by nestmate 2 towards nestmate 1 are assigned to *a*, all interactions initiated by nestmate 1 towards nestmate 2 are assigned to *b*, and so on. Nestmate discrimination was tested by looking for differences in tolerances between nestmates and non-nestmates. To do this tolerance indices T_a - T_f , corresponding to each of the categories *a-f* were calculated as shown in the example below:

$$T_a = \sum_{i=1}^n p_i r_i$$

where p_i is the proportion of the i th behaviour in *a*, r_i , the tolerance rank of the i th behaviour and n , the total number of kinds of behaviours seen in all experiments put together (modified from Venkataraman *et al.*¹²).

ASSAY



- 1 *Label* : self produced
Template: self based
-
- 2 *Label* : not self produced
Template: non self based
-
- 3 *Label* : non self produced
Template: self based
-
- 4 *Label* : self produced
Template: non self based
-
- 5 *Label* : not self produced
Template: non self based

LEGEND

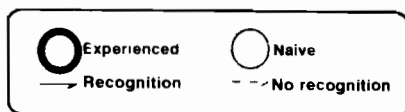


FIG. 6. An experimental design to determine the ontogeny of labels and templates used by wasps in nestmate discrimination. It is reasonable to expect that wasps discriminate nestmates from non-nestmates by detecting molecules on the surface of encountered wasps (let us call that Label) and comparing the label with a Template in their brains. As indicated in the upper box, we need an assay in which wasps which have been exposed to their nest and nestmates (which are therefore expected to have normal labels and templates irrespective of whether labels are self produced or acquired and templates are self based or non self based), can discriminate nestmates from non-nestmates. In Panel 1 isolated nestmates recognise each other. Thus labels must be self produced and templates must be self based. However, if, as in Panel 2, isolated nestmates cannot recognise each other then labels are not self produced and/or templates are non self based. To discover which of this is true or if both are true, we need to do experiments with one isolated and one exposed wasp, as in panels 3–5. In Panel 3, the exposed wasp cannot recognise its isolated nestmate but the isolated wasp recognizes its exposed nestmate. Therefore, the isolated wasp has the appropriate template but lacks the label. In other words, labels are non self produced but templates are self based. In Panel 4, the exposed wasp recognizes its isolated nestmate, but the isolated wasp cannot recognize its exposed nestmate. Hence the isolated wasp has the appropriate label but lacks the template. In other words, labels are self produced and templates are non self based. In Panel 5, neither can the exposed wasp recognize its isolated nestmate nor can the isolated wasp recognize its exposed nestmate. Clearly, the isolated wasps lack both the template and the label. In other words, labels are not self produced and templates are not self based (modified from Gadagkar¹¹).

Table IV
The mechanism of nestmate discrimination in
Ropalidia marginata (modified from Gadagkar²⁹)

<i>Discriminating wasps</i>	<i>Discriminated wasps</i>	<i>Discrimination</i>
Adults on nest	Adults on nest	Yes
Exposed	Exposed	Yes
Isolated	Isolated	No
Exposed	Isolated	No
Isolated	Exposed	No

discriminated wasps need to be exposed, for efficient discrimination to be possible, it suggests that the isolated wasps lack both the template and the label 11 (Fig. 6). Thus we concluded that both labels and templates are acquired/learned from a common external source, namely, the nest and/or nestmates. This means that all the wasps in a nest will have common labels and templates, making intra-colony kin recognition rather unlikely¹². Although we came to this conclusion by an indirect route, other investigators using related genera of social wasps have come to the same conclusion and sometimes by more direct methods^{13,14}. As far as we know, primitively eusocial wasps do not have well developed intra-colony kin recognition abilities to permit the kind of nepotism (to preferentially care for close relatives over more distant relatives) that can rescue the hypothesis that asymmetries in genetic relatedness created by haplodiploidy alone are sufficient to select for worker behaviour.

8. The search for factors other than genetic relatedness

The experiments on nestmate discrimination described above led us to move more confidently towards a research programme to investigate factors other than genetic relatedness that may tilt the cost benefit equation in favour of the worker strategy rather than the solitary nest-founding strategy. Lin and Michener¹⁵, who were perhaps the first investigators to explicitly consider factors other than genetic relatedness in moulding the evolution of insect societies argued that "social behavior in insects is in part mutualistic". The fact that nestmate discrimination occurs only when wasps are exposed and breaks down in the case of isolated wasps provided a convenient paradigm for us to study mutualistic interactions among wasps. In one experiment we used a nest built in a laboratory cage and studied interactions among wasps in the context of a nest. Into this cage we introduced exposed relatives, isolated relatives, exposed non-relatives as well as isolated non-relatives, none of which had ever encountered any of the wasps in the test cage. We collected one nest from one locality and cut it into three parts. One part was used to create the nest in the test cage and the remaining two parts were used to create exposed and isolated relatives of the test animals. A second nest was collected from a distant locality and cut into two parts which were used to create exposed and isolated non-relatives of the test animals (Fig. 7). Although none of the introduced wasps was allowed to join the nest, the behaviour of the resident wasps in the test cage towards the introduced wasps was most interesting. When the introduced wasps came close to the nest,

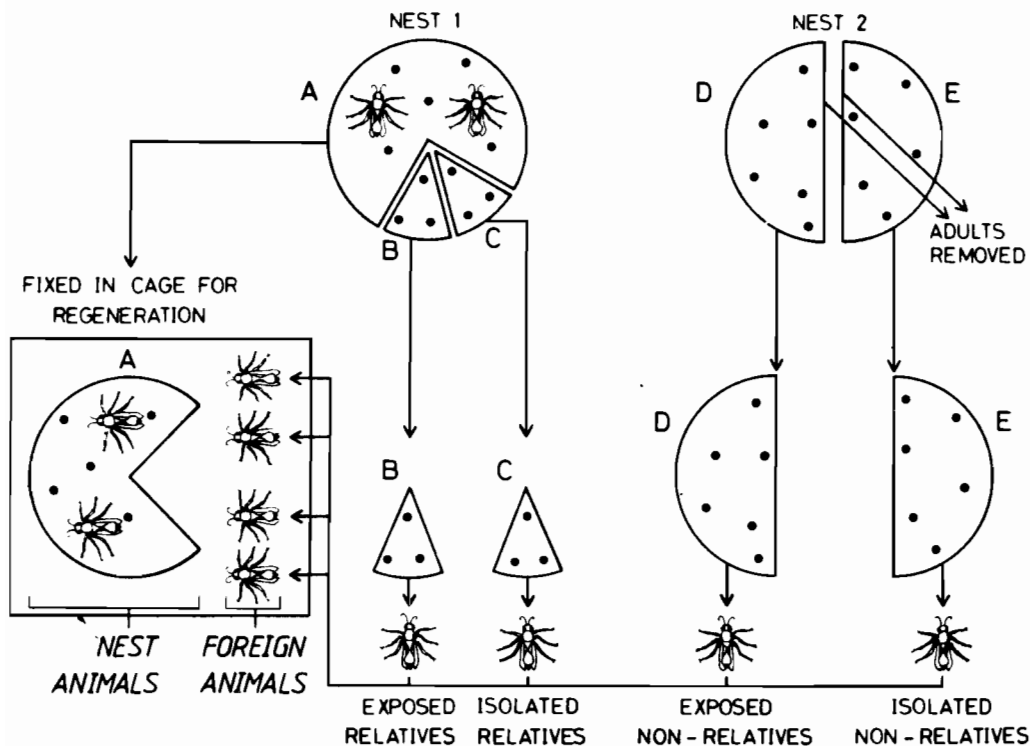


FIG. 7. An experimental design for exposing a colony of *R. marginata* to exposed relatives, isolated relatives, exposed non-relatives and isolated non-relatives of the resident wasps such that the introduced wasps have never before encountered the resident wasps. Nest 1 was cut into three parts. Part A was used to generate the test colony and parts B and C were used to obtain exposed relatives and isolated relatives of the wasps in the test colony. The exposed relatives were allowed to sit on their fragment of the nest for several days after their eclosion. However, since nest 1 was cut into three parts before the eclosion of any of the wasps, the exposed and isolated relatives had never encountered the wasps in the test colony. Note that the adult wasps present in the test colony included those present on nest 1 at the time of its collection as well as those that eclosed from fragment A after it was separated from fragments B and C. Nest 2 was cut into two parts to obtain exposed and isolated non-relatives of the wasps in the test colony. Nests 1 and 2 were collected from localities separated by at least 10 km (modified from Venkataraman³²).

the resident wasps treated exposed relatives more tolerantly than they did exposed or isolated non-relatives and treated isolated relatives in an intermediate way. This was consistent with our finding from experiments outside the context of the nest described above. But when the test animals encountered wasps away from the nest, they did not discriminate between different classes of introduced wasps¹⁶. This suggests that discrimination is context dependent and not merely based on genetic relatedness and that is what one would expect if the behaviour of the wasps is in part mutualistic rather than entirely altruistic.

9. How do wasps treat alien wasps?

In the experiment described above, none of the introduced wasps had any prior social experience as they had never lived in a normal colony. Also they were all 26 to 52 days old at the time of introduction. To make the experiment a little more natural, we now simply took all the wasps from one colony and introduced them into a cage containing a test colony which was unrelated to the colony providing the introduced wasps. The response was dramatically different from that of the previous experiment. The queen among the introduced wasps was singled out for violent aggression and was torn to pieces. Among the introduced workers, some were killed (albeit in a less dramatic manner), some were allowed to live in the cage without joining the nest and yet others were allowed to join the nest. The act of violently killing the queen is easy to interpret as she constitutes a direct reproductive threat to the resident colony.

But why were some wasps killed and others not, among those that were not allowed to join? Quite naturally we examined the behavioural patterns of the wasps in the original colony for a possible clue. Using time activity budgets of wasps in their original colonies, we found that the probability of being killed after introduction into an alien colony was negatively correlated with the proportion of time that the introduced wasps had spent away from their original colonies¹⁷ (Table V). This has several possible explanations. Wasps that spend much time away from their nests are usually foragers and the resident wasps may be more tolerant of aliens with proven track record in foraging and may even accept them into their nest under some situations. Wasps that are good foragers usually have relatively poorly developed ovaries and thus pose less of a reproductive threat to the resident wasps. Wasps that spend much time away from their nests may have less of their nest-specific smell and hence may be more acceptable to wasps from alien nests. Notice that these explanations are not necessarily mutually exclusive. Lack of a strong alien smell or lack of smell connected with developed ovaries may be the proximate mechanism that brings about the observed favourable treatment given to these wasps and the advantage of being tolerant of alien wasps that have poorly developed ovaries and/or that are good foragers may be the ultimate evolutionary advantage of such differential behaviour.

Table V
Why some alien wasps are killed ?
 (modified from Venkataraman and Gadagkar¹⁷)

<i>Variable</i>	<i>Estimated coefficients</i>	<i>Standard error</i>	<i>Z</i>
Absent from nest	-7.04	3.07	-2.29*
Sit & groom	2.62	2.24	1.17
Walk	15.46	8.66	1.92
Sit with raised antennae	1.40	5.11	0.28
In cell	3.91	11.40	0.34

* $p < 0.05$

10. Why are some wasps accepted into alien colonies?

But why were some alien wasps allowed to join the resident nest? All wasps which were allowed to join the nest were young (less than 8 days of age). Once again young wasps have poorly developed ovaries and thus pose little reproductive threat. Besides, the behaviour of young wasps can probably be moulded by the resident colony in ways that would benefit the colony. To investigate this phenomenon in more detail we performed a separate set of experiments where we systematically introduced wasps of different ages into caged colonies. Our results showed that wasps that are 8 days of age or less have a finite probability of being accepted while wasps older than 8 days of age have no chance of being accepted (Table VI). We also found that young wasps make more attempts to join the nest and are also more successful per attempt compared to older wasps¹⁸. But age and ovarian condition remain as confounding variables. In a new set of experiments we are now attempting to tease out the effects of age and ovarian condition.

In the meanwhile, the finding that young wasps are sometimes accepted into alien colonies suggested the possibility of another experimental paradigm. By introducing alien young wasps into other colonies we can create genetically mixed colonies. We took care to introduce young alien wasps from unrelated colonies so as to closely match the timing of eclosion of natal wasps in the test colonies. The aim of these experiments was to see if the genetically mixed colonies are in any way different from colonies with natural levels of genetic variability and if the introduced alien wasps are treated in any way differently by the resident wasps and by the wasps naturally eclosing on the test colonies. We have recently created 12 such genetically mixed colonies and made extensive behavioural observations both before and after the introduction of alien wasps. In spite of examining almost all behaviours shown by the wasps, we are unable to detect any differences between genetically mixed and genetically 'pure' colonies. Similarly, we are unable to detect any differences in the behaviour of introduced and natal wasps. The behavioural interactions between and within kin groups are also indistinguishable from each other. The introduced wasps also go on to become foragers and even queens in their foster colonies with probabilities not different from the probabilities with which natal wasps take on these roles. In short, there is no detectable effect of making a colony genetically mixed and the introduced, genetically unrelated, alien wasps become indistinguishable

Table VI
Why some alien wasps are accepted?
 (modified from Venkataraman and Gadagkar¹⁸)

<i>Variable</i>	<i>Estimated coefficients</i>	<i>Standard error</i>	<i>Z</i>
Intercept	687.86	1327.18	0.52
Colony 1	-683.94	1327.18	-0.52
Colony 2	-689.40	1332.78	-0.52
Colony 3	681.82	1327.18	-0.51
Colony 4	-688.21	1327.18	-0.52
Age at introduction	-0.86	0.13	-2.73*

* $p < 0.01$

from the natal wasps^{19, 20}. All these results reinforce the conclusion that factors other than genetic relatedness are more important in modulating the levels of cooperation and conflict in *R. marginata*.

Armed with these results, we have now begun to directly measure the cost, if any, of increased genetic variability. We have set up artificial laboratory nests with a pair of freshly eclosed wasps such that the members of the pair had either eclosed from the same nest or from very different nests. We then compare the productivities of the pure pairs and the mixed pairs. Our preliminary results suggest that there are no detectable differences between the productivities of genetically pure and mixed groups. If this trend persists we would be able to conclude that there is no detectable cost to such increased genetic variability²¹.

11. Why are some queens more successful than others?

There is however an intriguing and seemingly contradictory result that needs to be mentioned. In a long-term study of four colonies, we gathered data on the performance of 17 queens. The most striking feature of these data was the enormous variability in the performance of different queens. We measured queen success in four different ways: (i) length of tenure, (ii) total number of offspring produced, (iii) number of offspring produced per day of tenure, and (iv) the proportion of eggs laid that successfully become adults. We then explored the dependence of queen success on three potential determinants of queen success, namely, (a) age at take over, (b) mean worker: brood ratio during tenure and (c) mean worker-brood genetic relatedness during tenure. For each measure of queen success, we found a significant correlation with mean worker-brood genetic relatedness but not with the other two potential determinants (Table VII). The obvious interpretation of this finding is that queens are more successful when worker-brood genetic relatedness is high²². At first glance this seems to contradict our previous conclusion that kin recognition abilities are not well developed. However, it is entirely possible that while wasps can assess the overall level of their relatedness to the brood, they may still be unable to discriminate between close and distant relatives (adults and brood) within the nest. We may tentatively conclude therefore that while the overall genetic relatedness to the group may decide their levels of cooperation and conflict with the group as a whole, different genetic relatedness values with individual members of the group do not seem to

Table VII
Correlations between indicators and possible determinants of queen success (modified from Gadagkar et al.²²)

	Age	Worker: Brood ratio	Worker-Brood relatedness
Queen tenure	N. S.	N. S.	$p < 0.01$
#offspring	N. S.	N. S.	$p < 0.01$
# offspring/day	N. S.	N. S.	$p < 0.02$
Prop. of eggs that become adults	N. S.	N. S.	$p < 0.05$

N. S. = Not significant

modulate differential levels of cooperation and conflict with different members of the group. But there is no doubt that wasps do show different levels of cooperation and conflict with different members within a group. This must therefore be modulated by other factors.

12. Genetic relatedness may often be relatively unimportant in modulating cooperation and conflict

The conclusion that genetic relatedness may be relatively unimportant has also been reached by several other investigators. I will give three rather striking examples. Reeve²³ has modeled factors that favour solitary nesting, joining and nest usurpation in polistine wasps and has shown that solitary nesting and usurpation are favoured when ecological constraints on solitary nesting are weak and usurpation and joining are favoured when ecological constraints on solitary nesting are strong. Most interestingly, the parameter specifying genetic relatedness between co-foundresses drops out completely from his model, implying no significant role for genetic relatedness between co-foundresses in modulating cooperative behaviour (= joint nesting). If genetic relatedness between co-foundresses had a significant role in modulating the levels of cooperation and conflict, one would expect more multiple foundress nests and fewer single foundress nests in species where genetic relatedness between co-foundresses is high. On the contrary, Reeve²³ found a significantly negative correlation between genetic relatedness among co-foundresses and proportion of multiple foundress nests in eight species of *Polistes* and Hughes *et al.*²⁴ failed to find a positive correlation suggesting again that genetic relatedness is unlikely to be important in modulating the levels of cooperation and conflict in insect societies. Finally, Nonacs²⁵ has modeled the dynamics of colony founding in ant species which, somewhat like polistine wasps, start new colonies as multiple foundress associations where usually only one of the foundresses persists as the queen up until the time that the colony starts to produce future reproductives. His models show that for ants queens faced with the dilemma of whether to remain at the present nesting site or to leave in the hope of finding a better site, discriminating competitive ability among potential co-foundresses is more important than discriminating kinship.

13. All wasps are not equal

But what factors other than genetic relatedness might be important in modulating the levels of cooperation and conflict? One way to begin to unravel other potential factors is to recognize that an implicit assumption in all the foregoing analysis is that all wasps are equal. This assumption may not be true at all. Indeed we have evidence that there is a pre-imaginal caste-bias such that poorly nourished larvae are channeled into a developmental pathway that makes them more likely to become workers while better nourished larvae are channeled into a developmental pathway that makes them more likely to become queens²³⁻²⁵ (Fig. 8). It is easy to see that the propensities for cooperation and conflict will depend significantly on the inherent abilities of the wasps to be able to become queens or workers.

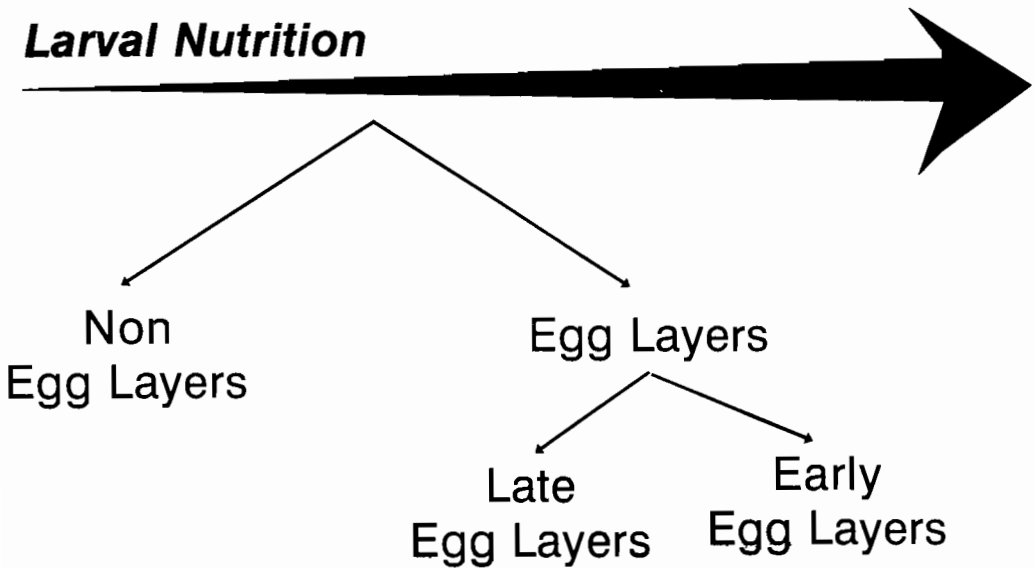


FIG. 8. When freshly eclosed female *R. marginata* wasps are isolated into laboratory cages and provided an *ad libitum* diet and adequate building material, only about 50% of them initiate nests and lay eggs while the other half die without doing so. Because these conditions are expected to simulate solitary nest founding, we conclude that there is a pre-imaginal caste bias. There is evidence that larval nutrition is the basis of such pre-imaginal caste bias such that poorly nourished larvae develop into non-egg layers and better nourished larvae develop into egg layers. There appears to be a further differentiation into early and late egg layers, also on the basis of larval nutrition (for details see Gadagkar *et al.*²⁶⁻²⁸).

14. Wasps are almost human!

I will end with an anecdote about wasps that will both help me keep my promise of showing that wasps very nearly approach humans in the richness and complexity of their interactions and will also point to ways of unraveling other factors that might modulate levels of cooperation and conflict. I was studying a colony of *Ropalidia cyathiformis*, a related social wasp with much the same biology and ecology, in early 1985²⁹. The purpose of my study was to record the behaviour of all the wasps in a colony and experimentally remove the queen to see who becomes the next queen. The hope was that by doing several such experiments, I would be able to predict who the next queen would be, even before the removal or death of the existing queen. It is another matter that in spite of numerous such experiments both with *R. cyathiformis* and *R. marginata*, we are unable to predict future queens with any accuracy^{30, 31}. This says a great deal about the complexity of the system.

Be that as it may, here was a situation where I had two wasps that were about equally aggressive and while I could guess that one of them would take over as the next queen if I removed the present one, I could not be sure which one might do so. As in all studies, I

had all the wasps, these two potential contenders for queenship, the present queen as well as six other workers, marked with unique spots of pints of different colour. The two wasps in question were marked with spots of blue and orange and let us simply call them Blue and Orange. When I removed the original queen, Blue became the next queen and somewhat to my surprise, Orange left the colony. This does not usually happen; when one of the workers take over as the replacement queen, the other workers stay on and work as the six other workers did on this occasion. However, the behaviour of the six workers who stayed on changed dramatically after Blue took over. All the six workers, who had worked normally during the tenure of the previous queen, immediately stopped working. They completely stopped foraging for food and building material. If they were absent from the nest, they would return with nothing. This 'non-cooperation' went on for over a week during which period Blue cannibalized on some of the eggs of the previous queen to make room for her own eggs. This was followed by the workers also cannibalizing on brood as they were obviously hungry. It seemed to me that the colony would be gradually abandoned due to such non-cooperation.

What happened instead was most dramatic. It turned out that Orange who appeared to have left after Blue took over as the queen, had not quite left. She would come back momentarily and I cannot help saying "as if to see how Blue was doing". Ten days later, Orange returned and took over as the new queen and Blue left. The behaviour of the same six workers changed dramatically once again. They seemed perfectly willing to cooperate with Orange. They brought food and fed the larvae and new cells were built for Orange to lay eggs. Even more dramatic was the fact that Blue had not quite left either. She also would come back momentarily as if to see how well Orange was doing! Eventually Blue seemed satisfied that Orange was doing very well and she returned to join the colony. But this could not be accomplished without the workers being very aggressive towards her and forcing her to sit a little away from the nest and be subordinated by them repeatedly. Clearly the same set of workers had the ability to respond so differently to the three successive queens and install one of their own choice. The ability of Blue and Orange to adjust to the changing situations is also most impressive. This incident suggests that the nature of inter-individual interactions among the wasps is sufficiently rich and complex to invite comparison with human social interactions. In addition, it also hints at cognitive abilities and inter-individual assessment, as another class of factors that might help mould the levels of cooperation and conflict in insect societies, factors that would be especially appropriate if insect societies are at least in part mutualistic!

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